Journal of Cellular Biochemistry 93:688–696 (2004)

Etiology of Paget's Disease and Osteoclast Abnormalities

Sakamuri V. Reddy*

Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Abstract Paget's disease of bone is a chronic focal skeletal disorder that affects up to 2–3% of the population over the age of 60 years. Paget's disease is primarily a disease of the osteoclast. The pathologic abnormality in patients with Paget's disease involves increased bone resorption by the osteoclasts, followed by abundant new bone formation that is of poor quality. Genetic linkage analysis indicated that 40% of patients with Paget's disease have an affected first degree relative and 1% of patients develop osteosarcoma. Paget's disease is an autosomal dominant trait with genetic heterogeneity. Recurrent mutations in the ubiquitin-associated (UBA) domain of sequestosome 1 (SQSTM1/p62) are identified in patients with Paget's disease. Osteoclasts and osteoclast precursors from patients with Paget's disease contain paramyxoviral transcripts and appear hyperresponsive to 1,25-$(OH)_2D_3$ and RANK ligand (RANKL). It has been suggested that the enhanced sensitivity of osteoclast precursors for 1,25-$(OH)_2D_3$ in Paget's disease results from increased expression of coactivators of vitamin D receptor (VDR). However, a cause and effect relationship for the paramyxoviral infection and SQSTM1/p62 gene mutations associated with this disease and osteoclast abnormalities are unclear. Therefore, the etiology of Paget's disease remains uncertain. J. Cell. Biochem. 93: 688–696, 2004.

Key words: Paget's disease; osteoclast; measles virus (MV); sequestosome (p62); tartrate resistant acid phosphatase (TRAP); RANK ligand (RANKL)

Sir James Paget first described Paget's disease of bone in 1877 as Osteitis Deformans, a chronic focal skeletal disease that can be monostotic or polyostotic. Paget's disease is the second most common metabolic bone disease and affects between 2% and 3% of the population over the age of 60. The disease been associated with deformity and enlargement of single or multiple bones among which, the skull, clavicles, long bones, and vertebral bodies are the most frequently involved [Paget, 1877]. Patients with Paget's disease are frequently asymptomatic, but approximately 10–15% of the patients have severe symptoms including bone pain, fractures, neurological complications due to spinal cord compression, deafness, and dental abnormalities. Paget's disease is a highly localized disease, and new lesions rarely develop during the course of the disease. Rather, lesions continue to progress in size unless treated. The primary pathologic abnormality in patients with Paget's disease is increased bone resorption, followed by abundant new bone formation. The bone that is formed is disorganized and of poor quality, resulting in bowing of the bone, stress fractures, and arthritis in joints contiguous to the involved bones. In addition, patients with Paget's disease can develop hypercalciuria and hypercalcemia, due to accelerated bone resorption induced by immobilization. Another interesting feature of Paget's disease is that bones not clinically involved with Paget's disease appear to show increased bone remodeling. This increased bone remodeling in unaffected bones has been ascribed to secondary hyperparathyroidism rather than to subclinical involvement of the bones with Paget's disease. However, less than 20% of patients with Paget's disease have elevated parathyroid hormone (PTH) levels [Siris, 1999]. A juvenile form of Paget's disease also called hyperostosis corticalis deformans juvenilis or hereditary hyperphosphatasia is very different than the adult form of

Grant sponsor: National Institutes of Health; Grant numbers: DE 12603, AR 049363; Grant sponsor: DOD Medical Research Award.

*Correspondence to: Sakamuri V. Reddy, PhD, Director of the Osteoclast Center, Children's Research Institute, Department of Pediatrics, Medical University of South Carolina (MUSC), 135 Rutledge Avenue, P.O. Box. 250561, Charleston, SC 29425. E-mail: reddysv@musc.edu

Received 30 June 2004; Accepted 1 July 2004

DOI 10.1002/jcb.20256

the disease. It is characterized by widespread involvement of the skeleton and has histological and radiological features that distinguish it from Paget's disease of the adults, including the absence of viral-like nuclear inclusions in osteoclasts present in the bone microenvironment [Whyte et al., 2002].

Bone scans are the most sensitive method of detecting pagetic lesions and can be used to follow the activity of the disease in these patients. Initial lesions appear osteolytic, followed by a chaotic sclerotic appearance, and finally become osteosclerotic. Considerable thickening of the sclerotic bone results in bone deformity. Serum tartrate resistant acid phosphatase (TRAP), presumably released by osteoclasts, appears to be an index of bone resorption in Paget's disease but is not routinely used. The most useful markers for the increased osteoblast activity in Paget's disease are the total alkaline phosphatase and bone-specific alkaline phosphatase activity levels in serum. Patients also showed significantly higher endothelin-1 circulating levels than controls with a positive correlation with serum alkaline phosphatase, but not with urinary hydroxyproline [Tarquini et al., 1998]. Furthermore, serum calcium levels are typically normal in Paget's disease and also serum osteocalcin levels appear to be a poor index of the progression of the disease. Bisphosphonates are the most common treatment for patients with Paget's disease. These inorganic phosphate compounds inhibit osteoclast-mediated bone resorption and induce osteoclast apoptosis [Siris, 1999]. It has been reported that serum M-CSF levels are significantly elevated in patients with Paget's disease, however not significantly different in patients under treatment compared to normal subjects [Neale et al., 2002].

Paget's disease has a very unusual geographic distribution, with an increased incidence in Caucasians of European descent, but it also occurs in African Americans. It is rare in those of Asian descent. Studies also suggested high prevalence rates of radiographic Paget's disease in Britain, Australia, North America, and Western Europe. Interestingly, even within Britain there is a marked geographic variation in the incidence of Paget's disease, with an increased incidence in the Western portion of England and a much lower incidence in the Southern portion of England (8% vs. 4%) [Cooper et al., 1999]. The prevalence of the disease is 2.5% among men and 1.6 % among women aged 55 years and above in British towns. The level of prevalence in Spain is estimated to be at 1.5%. A radiographic survey of 24 patients with Paget's disease in Ireland further revealed monostotic disease in 8 and polyostotic disease in 16. There have been reports that one to three million patients over the age of 55 are affected with Paget's disease in the United States. This is in contrast with the extreme rarity of the disease in Scandinavia, Ireland, and Southern Europe. This unusual geographic distribution for the incidence of Paget's disease is not attributable to geographic, environmental, or industrial exposures in these areas and currently cannot be explained. Furthermore, the incidence of Paget's disease appears to be decreasing over the last several decades [Siris, 1999; Doyle et al., 2002], but the basis for this decrease in the incidence of Paget's disease is unknown. However, it is evident that genetic factors play important role in the familial and sporadic forms of Paget's disease. Furthermore, Paget's disease has been described as a slow paramyxoviral infection process, suggesting a viral etiology for the disease. Therefore, this review will focus on the etiology of Paget's disease with an emphasis on the role that genetic and paramyxoviral infection may play in abnormal osteoclast development responsible for excess bone resorption in patients with Paget's disease.

GENETICS OF PAGET'S DISEASE

Familial incidence is common in Paget's disease and 40% of patients with the disease have an affected first-degree relative. Therefore, genetic factors play an important role in the pathogenesis of Paget's disease of bone. The disease often is inherited in an autosomal dominant manner manifesting genetic heterogeneity and incomplete penetrance. Familial Paget's disease has an equal incidence in males and females. A genetic locus for Paget's disease has been identified on chromosome 18q [Leach et al., 2001; Good et al., 2002] in several large families with Paget's disease in a region near the familial expansile osteolysis (FEO) locus. FEO is a disease related to Paget's disease but occurring in patients at a much younger age and being a much more severe disease. FEO is an extremely rare disease, affecting only a very limited number of kindreds in the world, also mapped to chromosome 18q and is linked to activating mutations in the TNFRSF11A gene

which encodes receptor activator of nuclear factor κB (RANK) [Hughes et al., 2000]. Recently, in patients with Juvenile Paget's disease, a homozygous deletion of the gene on chromosome 8q24.2 that encodes osteoprotegerin, member of the superfamily of tumor necrosis factor receptors, has been reported [Whyte et al., 2002]. However, linkage studies, coupled with mutation screening have excluded involvement of RANK and also osteoprotegerin in the majority of patients with Paget's disease of bone [Sparks et al., 2001]. Studies also indicated that patients with Paget's disease have an increased incidence of osteosarcoma, with approximately 1% of patients with Paget's disease developing osteosarcoma in an affected bone. This incidence of osteosarcoma is 1,000 times higher than that in the general population for this age group. Recent genetic studies have demonstrated linkage in seven of seven patients with osteosarcoma to loss of heterozygosity in a region of 18q that is adjacent to or within a locus for Paget's disease on 18q [Hansen et al., 1999].

A genome wide search in familial Paget's disease of bone further indicated genetic heterogeneity of the disease with candidate loci on chromosomes 2q, 10q, and 5q [Hocking et al., 2001]. More recently, the gene encoding sequestosome 1 (SQSTM1/p62) mapped within the critical region on chromosome 5q35-qter identified a proline-leucine amino acid change at codon 392 (P392L) in French-Canadian patients with Paget's disease of bone [Laurin et al., 2002]. The frequency of mutation was 16% and 46% for sporadic and familial cases tested, respectively. Further studies also identified different mutations affecting the highly conserved ubiquitin-binding domain of SQSTM1/p62 protein in patients with familial and sporadic Paget's disease [Johnson-Pais et al., 2003; Good et al., 2004; Hocking et al., 2004].

ROLE OF SQSTM1/P62 IN OSTEOCLASTOGENESIS

The atypical PKC (aPKC) interaction with SQSTM1/p62 has been implicated in signaling cascades that control NF-κB activation. It is evident that p62 provides a scaffold linking the aPKCs to the tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) receptor signaling complexes through its interaction with RIP and TRAF-6, respectively [Moscat and Diaz-Meco, 2000]. Thus, SQSTM1/p62 mediate IL-1 and TNF-α cytokine signaling to activate NF-κB (Fig. 1). TRAF-6 plays an essential role in RANK ligand (RANKL) signaling during osteoclastogenesis. More recently, it has been shown that RANKL stimulation results in upregulation of p62 expression in osteoclast precursor cells and that the genetic inactivation of p62 in mice impaired PTHrP induced osteoclastogenesis in vivo. However, p62 null mice have grossly normal skeletal phenotype and that no alterations were found in the trabecular size and number of osteoclasts compared to wild-type mice. In vitro studies further demonstrated that p62 deficiency leads to inhibition of IKK activation and NF-κB nuclear translocation during osteoclastogenesis [Duran et al., 2004]. These studies also demonstrated that RANKL stimulation induces formation of a ternary complex involving TRAF-6, p62, and aPKC during osteoclastogenesis. Recent studies also identified novel mutations in ubiquitin-associated (UBA) domain of SQSTM1/p62, however, genotype-phenotype analysis indicated that there is no correlation with respect to different mutations in UBA and disease occurrence [Hocking et al., 2004]. Therefore, the precise role that SQSTM1/p62 may play in the pathogenesis of Paget's disease of bone remains to be elucidated.

VIRAL ETIOLOGY

Since the early 1970s, a variety of studies have implicated paramyxoviruses in Paget's disease. The viral etiology has been proposed for Paget's disease with an initial description of nucleocapsid-like structures in the nuclei and cytoplasm of pagetic osteoclasts by electron microscopy [Mills and Singer, 1976]. Immunocytochemical studies further confirmed that these nuclear inclusions cross-reacted with antibodies that recognized measles virus (MV) or respiratory syncytial virus (RSV) nucleocapsid antigens. In situ hybridization techniques also identified the presence of MV messenger RNA (mRNA) sequences in up to 90% of osteoclasts and other mononuclear cells in pagetic bone specimens. Similarly, canine distemper virus (CDV) nucleocapsid antigens were also detected in osteoclasts from patients with Paget's disease. These paramyxoviral-like nuclear inclusions are not unique to Paget's disease and were reported in patients with FEO and rarely in patients with osteopetrosis, pycnodysostosis, and otosclerosis, oxalosis [Singer, 1999]. This has raised the

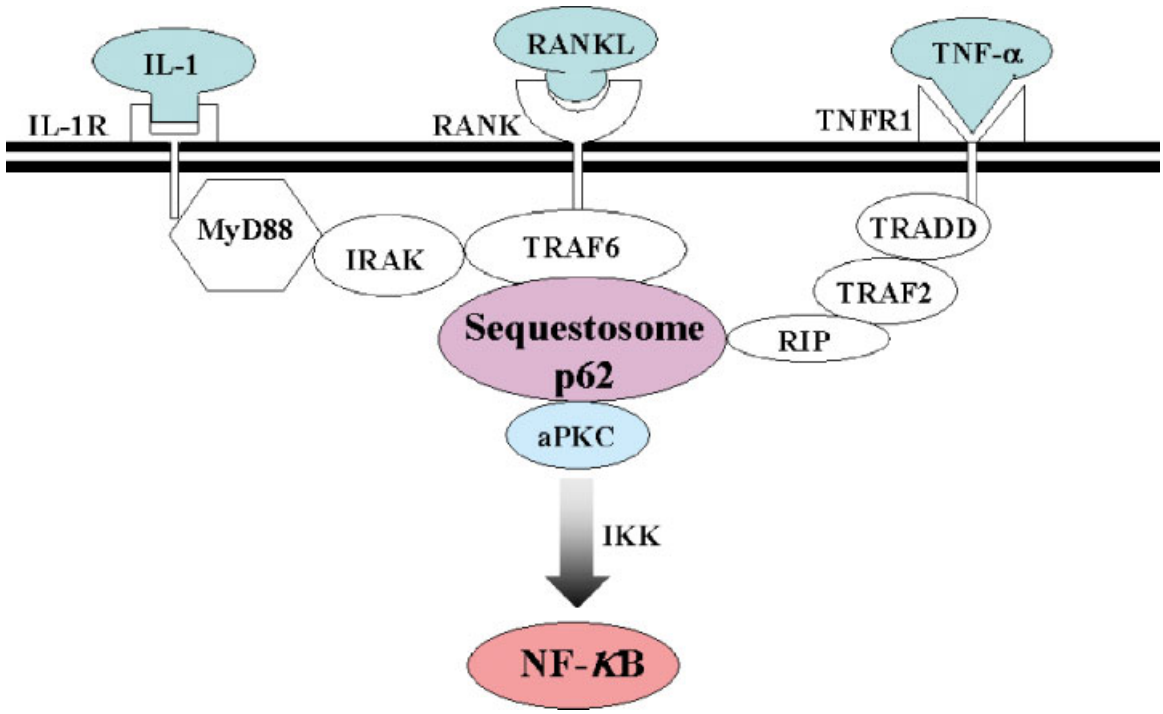

Fig. 1. Signaling cascades associated with sequestosome/p62. Sequestosome provides a scaffold linking the aPKCs to the TNF-α and IL-1 receptor signaling complexes through its interactions with RIP and TRAF-6, respectively resulting in phosphorylation of IKK and activation of NF-κB. RANKL-RANK signaling induce p62 to form a ternary complex with TRAF-6 and aPKCs during osteoclast differentiation [Duran et al., 2004]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

possibility that that the virus may be a non-etiologic agent in a cell altered by a genetic defect.

To further explore the viral etiology, using reverse transcription-polymerase chain reaction (RT-PCR) analysis, we have amplified the MV nucleocapsid (MVNP) transcripts from freshly isolated bone marrow cells from patients with Paget's disease. These MVNP transcripts contain mutations clustered at the c-terminal end of the mRNA [Reddy et al., 1995]. All these mutations were sense mutations and resulted in amino acid substitutions in the nucleocapsid gene product. The mutations occurred at 1% rate in the total MVNP gene isolated from a patient with Paget's disease. We further demonstrated that osteoclast precursors, the granulocyte macrophage colony-forming unit (CFU-GM), as well as mature osteoclasts from patients with Paget's disease, expressed MVNP transcripts. Since CFU-GM circulate and also give rise to monocytes and granulocytes in the peripheral blood, we then examined peripheral blood mononuclear cells from patients with Paget's disease and normals for expression of MVNP transcripts. We found by RT-PCR analysis that peripheral blood samples from 9 of 10 patients with Paget's disease contain MVNP transcripts, while none of the 10 normals tested expressed MVNP transcripts [Reddy et al., 2001a]. We were unable to find CDV or RSV nucleocapsid transcripts in patients we have studied. In contrast, CDV nucleocapsid transcripts were detected in affected bones from 100% of patients tested using in situ RT-PCR techniques. Furthermore, it has also been demonstrated that infecting canine bone marrow cells with CDV results in development of multinucleated cells that share some of the phenotypic characteristics of pagetic osteoclasts [Gordon et al., 1992]. However, other workers have been unable to detect paramyxoviral nucleocapsid transcripts in samples obtained from patients with Paget's disease [Helfrich et al., 2000; Ooi et al., 2000].

The presence of MV or CDV transcripts in osteoclasts and osteoclast precursors from patients with Paget's disease does not infer a

pathophysiologic role for these genes in the development of the pagetic lesions. It is possible that these paramyxoviral transcripts and paramyxoviral-like inclusions are simply markers for the disease and have no pathophysiologic significance. In studies, using normal osteoclast precursors (CFU-GM) transduced with retroviral vectors expressing the MVNP gene formed large osteoclasts more rapidly with an increased numbers of nuclei, hypersensitive to 1,25-dihydroxyvitamin D3 (1,25-$[OH]_2D_3$) and had increased bone resorbing capacity compared to normal osteoclasts. In contrast, normal osteoclast precursors transduced with the MV matrix gene did not express an abnormal phenotype [Kurihara et al., 2000]. In further studies, we have targeted CD46, human MV receptor to cells of the osteoclast lineage in transgenic mice and demonstrated that MV infection of osteoclast precursors from CD46 transgenic mice form osteoclasts, which express a pagetic phenotype in vitro [Reddy et al., 2001b]. Taken together, these data suggest a potential pathophysiologic role for the paramyxoviral nucleocapsid gene that is expressed in patients with Paget's disease. Mouse models of MV infection were also developed in which CD46 is introduced into transgenic mice and has been bred to another transgenic mouse lacking the alpha-beta-interferon receptor. Upon exposure to MV, these mice developed immune-suppression similar to patients with acute MV infection. The mice lack the alpha-beta interferon receptor demonstrated persistence of MV infection for at least 12 days [Peng et al., 2003]. However, TRAP-CD46 mice do not develop sustained MV infection, most likely reflecting the need for blocking interferon production for development of persistent MV infection in these mice.

MV infection has a similar incidence worldwide and occurs in very young patients, whereas Paget's disease is a disease of the elderly. These observations suggest that if paramyxoviruses have an etiologic role in Paget's disease, these viral infections must persist for long periods of time. To further investigate a potential site for the initial infection of osteoclast precursors with Paget's disease, we tested the hypothesis that very early pluripotent hematopoietic stem cells, which can persist for long periods of time in a quiescent phase, may be the initial target for the paramyxoviral infection in patients with Paget's disease. We found that other hematopoietic lineages from patients with Paget's disease in addition to the osteoclast lineage, including the erythroid and the erythroid precursors, burst-forming unit-erythroid (BFU-E), and multipotent myeloid precursors (CFU-GEMM), which give rise to megakaryocytes, monocytes, erythroid cells, and granulocytes, also contain paramyxoviral nucleocapsid transcripts [Reddy et al., 2001a]. Thus, if the initial site of infection occurs in a small number of primitive pluripotent hematopoietic stem cells that predominantly remain in Go, this might explain the chronicity of the infection. Furthermore, there may be a genetic predisposition for chronic paramyxoviral infections of hematopoietic precursors in patients with Paget's disease. However, a cause and effect relationship of paramyxoviruses in Paget's disease remains proven as yet no infectious virus been isolated from pagetic cells and also, it is not clear how the initial lesion occurs in Paget's disease.

PAGETIC OSTEOCLASTS

Histologic examination of pagetic bone biopsy revealed abundant structurally abnormal osteoclasts. Osteoclasts are increased in number and size, and contain as many as 100 nuclei per multinucleated cell compared to three to five nuclei for a normal osteoclast. These osteoclasts have characteristic ultrastructural abnormalities including microfilaments, paracrystalline arrays located in the nucleus and sometimes in the cytoplasm that are absent in non-pagetic bone or bone marrow cells. These inclusions closely resemble nucleocapsids of viruses of the paramyxoviridae family [Mills and Singer, 1976]. Osteoblasts are also increased in lesions in patients with Paget's disease, and they appear to be morphologically normal. Osteoblasts contain abundant rough endoplasmic reticulum and mitochondria in a well-developed Golgi zone, consistent with the increased bone formation activity that occurs in the active lesions. In advanced lesions in patients with Paget's disease, the marrow is also abnormal. The bone matrix in Paget's disease is highly abnormal in structure due to disordered bone remodeling. The bone matrix consists of erratic patterns of "cement lines" and demonstrates a "mosaic" pattern. The matrix is interspersed with numerous foci of woven bone, reflecting the increased rates of bone deposition that is of poor quality.

The bone marrow culture techniques identified several abnormalities in osteoclast formation and osteoclast precursors from patients with Paget's disease. Osteoclast-like multinucleated cells formed more rapidly with increased numbers (10–100-fold) and nuclei per osteoclast, expressed high levels of TRAP in marrow cultures from patients with Paget's disease compared to normals. In addition, osteoclast formation in pagetic bone marrow cultures was induced at concentrations of 1,25-$(OH)_2D_3$ that were 10–100 times lower than those required in normal marrow cultures. Structural examination of the osteoclast-like cells formed in bone marrow cultures also showed that they had many of the features of pagetic osteoclasts but lacked the characteristic nuclear and cytoplasmic inclusions. Immunocytochemical studies confirmed that MV and RSV nucleocapsid antigens were expressed in osteoclasts formed in vitro in these cultures. Osteoclasts from patients with Paget's disease also appear to produce increased levels of IL-6 and express high levels of IL-6 receptors compared to normal osteoclasts. In situ hybridization studies have further identified increased levels of IL-6, c-fos proto-oncogene, Bcl 2 anti-apoptotic gene mRNA expression in pagetic osteoclasts. IL-6 receptor and NF-IL-6 mRNA levels were also increased in osteoclasts from bone samples from patients with Paget's disease compared to those with osteoarthritis [Hoyland et al., 1994]. These data suggest that IL-6, which is a stimulator of human osteoclast formation, may act as an autocrine/paracrine factor to enhance osteoclast formation in patients with Paget's disease and increase the osteoclast precursor pool. IL-6 levels were also shown to increase in bone marrow plasma and peripheral blood of patients with Paget's disease [Roodman et al., 1992]. In addition, the increased levels of IL-6 in the peripheral blood of patients with Paget's disease may in part explain the increased bone remodeling seen in bones not clinically involved with Paget's disease.

To further investigate the potential abnormalities in osteoclast precursors in patients with Paget's disease, the number of osteoclast precursors in marrow aspirates from involved bones from patients with Paget's disease were assessed. It has been found that the number of early osteoclast precursors, CFU-GM, was increased significantly in marrow aspirates from patients with Paget's disease compared to normals. Interestingly, when the osteoclast precursors were separated from the marrow microenvironmental elements present in the marrow aspirates, similar numbers of osteoclast precursors were detected in these aspirates. These data suggested that the marrow microenvironment enhanced osteoclast precursor growth compared to the normal marrow microenvironment.

To determine the potential role of the marrow microenvironment and the enhanced osteoclast formation in patients with Paget's disease, reconstitution experiments were conducted using highly purified populations of osteoclast precursors from patients with Paget's disease or normals and marrow stromal cells from patients with Paget's disease and normals. Coculture of normal osteoclast precursors with marrow stromal cells from patients with Paget's disease resulted in enhanced growth of the osteoclast precursors from normals. Interestingly, when osteoclast precursors from patients with Paget's disease were cocultured with marrow stromal cells from normals, they also showed increased growth. These data suggest that both the marrow microenvironment, as well as the osteoclast precursors, are abnormal in patients with Paget's disease.

These studies also confirmed that the osteoclast precursors were hypersensitive to 1,25-$(OH)_2D_3$ compared to normals. The increased sensitivity of osteoclast precursors from Paget's patients to 1,25-$(OH)_2D_3$ is mediated through the vitamin D3 receptor (VDR). This was confirmed by upregulation of 24-hydroxylase mRNA expression in pagetic osteoclast precursors at concentrations of 1,25-$(OH)_2D_3$ that are one log less than that required for normal osteoclast precursors. The increased sensitivity to 1,25-$(OH)_2D_3$ was not due to increased numbers of vitamin D receptors in pagetic osteoclast precursors compared to normals, but appeared to be due to enhanced affinity of the VDR in pagetic cells for its ligand compared to normals [Menaa et al., 2000a]. Recently, it has been demonstrated that MVNP gene expression in osteoclast precursors results in increased levels of TAF_{II}-17 transcription factor gene expression. The high levels of TAF_{II}-17 permit formation of VDR transcription complex at low levels of receptor occupancy by 1,25-$(OH)_2D_3$ [Kurihara et al., 2004]. These results support the hypothesis that part of the pathophysiology underlying the increased osteoclast activity in Paget's

disease is due to increased levels of VDR coactivators that enhance VDR-mediated gene transcription at low levels of 1,25-$(OH)_2D_3$. These studies suggested that Paget's disease may be a VDR coactivator disease.

The osteoclast precursors from patients with Paget's disease also appear to be hyperresponsive to receptor activator of NF-κB ligand (RANKL) and that marrow stromal cells from pagetic lesions have increased RANKL expression [Neale et al., 2000; Menaa et al., 2000b]. RANKL is a critical osteoclast differentiation factor that is expressed on marrow stromal and osteoblast cells in response to several osteotropic factors. The increased sensitivity of osteoclast precursors from Paget's patients to RANKL appears to be due to interactions of these precursors with interleukin-6 (IL-6). Addition of neutralizing antibodies to IL-6 decreased the sensitivity of the osteoclast precursors from patients with Paget's disease to RANKL to normal levels. Similarly, addition of IL-6 to cultures of normal osteoclast precursors enhanced the responsivity of these precursors to RANKL to the levels seen with pagetic osteoclast precursors. Pagetic osteoclasts expressing MVNP gene produce high levels of cytokines that increase osteoclast precursor pool as well as osteoblast precursor proliferation and constitutive expression of RANKL, which contribute to the abnormal osteoclast development and highly localized nature of Paget's disease (Fig. 2). Immature osteoblasts are the major responders to RANKL inducing cytokines and studies also suggested that expression of RANKL decreases with osteoblast maturation [Gori et al., 2000]. Therefore, the increased numbers of highly active osteoblasts rapidly form large amounts of woven bone in patients with Paget's disease. Furthermore, the prodigious amounts of cytokine production by the pagetic osteoclasts result in continued stimulation of osteoblast precursors growth making the local microenvironment in the pagetic lesion progressively more osteoclastogenic resulting in elevated levels of bone resorption in these patients. Consistent with this hypothesis are findings of high levels of cytokines such as IL-6 being produced by pagetic osteoclasts and the increased RANKL protein levels in marrow adherent cells from pagetic lesions compared to normal marrow and uninvolved bones from the same patient [Menaa et al., 2000b]. In addition, the clinical observation that inhibiting osteoclast formation with bisphosphonates results in a dramatic fall in alkaline phosphatase in

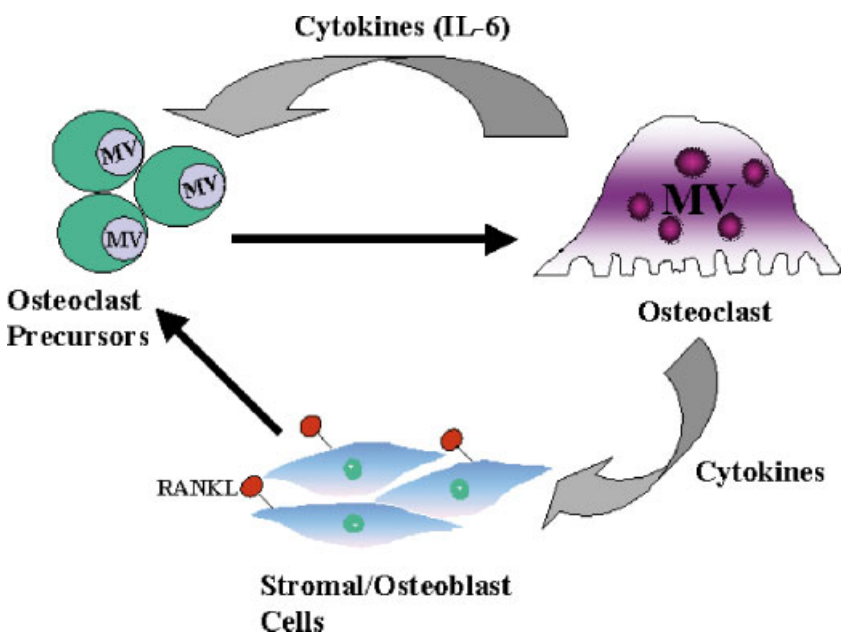

Fig. 2. Osteoclastogenesis in pagetic bone microenvironment. The osteoclast precursors contain measles virus (MV) transcripts and are hyperresponsive to RANK ligand (RANKL). The pagetic osteoclasts produce increased levels of cytokines such as IL-6, which enhance osteoclast formation. Chronic exposure to cytokines produced by the pagetic osteoclasts results in constitutive overexpression of RANKL in stromal/osteoblast cells further enhancing the abnormal osteoclast development in pagetic bone lesions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

patients with Paget's disease, demonstrates that osteoclasts are driving the osteoblastic response in Paget's disease. Alternatively, pagetic osteoclasts may live for very long periods of time compared to normal osteoclasts and persist in the lesion. It has been reported that the anti-apoptotic gene Bcl-2 is overexpressed in pagetic osteoclasts, suggesting that the osteoclasts lifespan may be prolonged in pagetic lesions. More recently, it has also been reported that mutations in the Bcl-2 gene promoter are responsible for upregulation of Bcl-2 expression leading to enhanced osteoclastogenesis in patients with Paget's disease [Brandwood et al., 2003]. Recently, it has been shown that SHIP, inositol 5′ phosphatase deficient mice are severely osteoporotic with an increased numbers of osteoclast precursors and hyperactive osteoclasts. In addition, serum levels of IL-6 are markedly increased in these mice as in Paget's disease [Takeshita et al., 2002]. However, the basis for these abnormalities in both osteoclasts and osteoclast precursors from patients with Paget's disease is still unknown.

SUMMARY AND FUTURE DIRECTIONS

Paget's disease of bone is the second most disorder of bone after osteoporosis. The disease is an autosomal dominant trait with genetic heterogeneity. The recent discovery of recurrent mutations occurring in the UBA domain of SQSTM1/p62 in patients with Paget's disease suggests that genetic factors may play an important role. Although SQSTM1/p62 mutations are implicated as a common cause of familial and sporadic Paget's disease, there is no correlation among different mutant forms of p62 and disease severity. Lack of skeletal abnormalities in p62 null mice further suggests a potential role for genes present in other candidate loci that were linked with Paget's disease. Alternatively, a genetic defect may favor the environmental factors such as MV infection to have potential role in pathogenesis of the disease. However, the molecular basis for the abnormalities associated with osteoclasts, the role of paramyxoviral infection and persistence of the virus in patients with Paget's disease is unclear. Targeting the expression of candidate genes to the cells of osteoclast lineage in transgenic mouse that are permissive to MV infection may allow better understanding of the pathobiology of Paget's disease. It is important to determine a cause and effect relationship for persistence of paramyxoviral infection and genetic predisposition in these patients. Therefore, the etiology of Paget's disease remains uncertain.

REFERENCES

Brandwood CP, Hoyland JA, Hillarby MC, Berry JL, Davies M, Selby PL, Mee AP. 2003. Apoptotic gene expression in Paget's disease: A possible role for Bcl-2. J Pathol 201:504–512.

Cooper C, Schafheutle K, Dennison E, Kellingray S, Guyer P, Barker D. 1999. The epidemiology of Paget's disease in Britain: Is the prevalence decreasing? J Bone Miner Res 14:192–197.

Doyle T, Gunn J, Anderson G, Gill M, Cundy T. 2002. Paget's disease in New Zealand: Evidence for declining prevalence. Bone 31:616–619.

Duran A, Serrano M, Leitges M, Flores JM, Picard S, Brown JP, Moscat J, Diaz-Meco MT. 2004. The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. Dev Cell 6:303–309.

Good DA, Busfield F, Fletcher BH, Duffy DL, Kesting JB, Andersen J, Shaw JT. 2002. Linkage of Paget disease of bone to a novel region on human chromosome 18q23. Am J Hum Genet 70:517–525.

Good DA, Busfield F, Fletcher BH, Lovelock PK, Duffy DL, Kesting JB, Andersen J, Shaw JT. 2004. Identification of SQSTM1 mutations in familial Paget's disease in Australian pedigrees. Bone 35:277–282.

Gordon MT, Mee AP, Anderson DC, Sharpe PT. 1992. Canine distemper virus transcripts sequenced from pagetic bone. Bone Miner 19:159–174.

Gori F, Hofbauer LC, Dunstan CR, Spelsberg TC, Khosla S, Riggs BL. 2000. The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. Endocrinol 141:4768–4776.

Hansen MF, Nellissery MJ, Bhatia P. 1999. Common mechanisms of osteosarcoma and Paget's disease. J Bone Miner Res 14:39–44.

Helfrich MH, Hobson RP, Grabowski PS, Zurbriggen A, Cosby SL, Dickson GR, Fraser WD, Ooi CG, Selby PL, Crisp AJ, Wallace RG, Kahn S, Ralston SH. 2000. A negative search for a paramyxoviral etiology of Paget's disease of bone: Molecular, immunological, and ultrastructural studies in UK patients. J Bone Miner Res 15:2315–2329.

Hocking LJ, Herbert CA, Nicholls RK, Williams F, Bennett ST, Cundy T, Nicholson GC, Wuyts W, Van Hul W, Ralston SH. 2001. Genomewide search in familial Paget's disease of bone shows evidence of genetic heterogeneity with candidate loci on chromosome 2q36, 10q13 and 5q35. Am J Hum Genet 69:1055–1061.

Hocking LJ, Lucas GJ, Daroszewska A, Cundy T, Nicholson GC, Donath J, Walsh JP, Finlayson C, Cavey JR, Ciani B, Sheppard PW, Searle MS, Layfield R, Ralston SH. 2004. Novel UBA domain mutations of SQSTM1 in Paget's disease of bone: Genotype phenotype correlation, functional analysis, and structural consequences. J Bone Miner Res 19:1122–1127.

Hoyland JA, Freemont AJ, Sharpe PT. 1994. Interleukin-6, IL-6 receptor, and IL-6 nuclear factor gene expression in Paget's disease. J Bone Miner Res 9:75–80.

Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RGH, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM. 2000. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet 24:45–48.

Johnson-Pais TL, Wisdom JH, Weldon KS, Cody JD, Hansen MF, Singer FR, Leach RJ. 2003. Three novel mutations in SQSTM1 identified in familial Paget's disease of bone. J Bone Miner Res 18:1748–1753.

Kurihara N, Reddy SV, Menaa C, Anderson D, Roodman GD. 2000. Osteoclasts expressing the measles virus nucleocapsid gene display a pagetic phenotype. J Clin Invest 105:607–614.

Kurihara N, Reddy SV, Araki N, Ishizuka S, Ozono K, Cornish J, Cundy T, Singer FR, Roodman GD. 2004. Role of TAFII-17, a VDR binding protein, in the increased osteoclast formation in Paget's disease. J Bone Miner Res 19:1154–1164.

Laurin N, Brown JP, Morissette J, Raymond V. 2002. Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget's disease of bone. Am J Hum Genet 70:1582–1588.

Leach RJ, Singer FR, Roodman GD. 2001. The genetics of Paget's disease of the bone. J Clin Endocrinol Metab 86:24–28.

Menaa C, Reddy SV, Barsony J, Cornish J, Cundy T, Roodman GD. 2000a. 1,25 dihydroxy-vitamin D3 hypersensitivity of osteoclast precursors from patients with Paget's disease. J Bone Miner Res 15:1–9.

Menaa C, Reddy SV, Kurihara N, Maeda H, Anderson D, Cundy T, Cornish J, Singer FR, Bruder JM, Roodman GD. 2000b. Enhanced RANK ligand expression and responsivity of bone marrow cells in Paget's disease of bone. J Clin Invest 105:1833–1838.

Mills BG, Singer FR. 1976. Nuclear inclusions in Paget's disease of bone. Science 194:201–202.

Moscat J, Diaz-Meco MT. 2000. The atypical protein kinase Cs. EMBO Rep 1:399–403.

Neale SD, Smith R, Wass JAH, Athanasou NA. 2000. Osteoclast differentiation from circulating mononuclear precursors in Paget's disease is hypersensitive to 1,25-dihydroxyvitamin D3 and RANKL. Bone 27:409–416.

Neale SD, Schulze E, Smith R, Athanasou NA. 2002. The influence of serum cytokines and growth factors on osteoclast formation in Paget's disease. QJM 95:233–240.

Ooi CG, Walsh CA, Gallagher JA, Fraser WD. 2000. Absence of measles virus and canine distemper virus transcripts in long-term bone marrow cultures from patients with Paget's disease of bone. Bone 27:417–421.

Paget J. 1877. On a form of chronic inflammation of bones (osteitis deformans). Med Chir Tr 60:37–63.

Peng KW, Frenzke M, Myers R, Soeffker D, Harvey M, Greiner S, Galanis E, Cattaneo R, Federspiel MJ, Russell SJ. 2003. Biodistribution of oncolytic measles virus after intraperitoneal administration into Ifnar-CD46Ge transgenic mice. Hum Gene Ther 14:1565–1577.

Reddy SV, Singer FR, Roodman GD. 1995. Bone marrow mononuclear cells from patients with Paget's disease contain measles virus nucleocapsid messenger ribonucleic acid that has mutations in a specific region of the sequence. J Clin Endocrinol Metab 80:2108–2111.

Reddy SV, Kurihara N, Menaa C, Roodman GD. 2001a. Paget's disease of bone: A disease of the osteoclast. Rev Endocr Metab Disord 2:195–201.

Reddy SV, Kurihara N, Menaa C, Landucci G, Forthal DF, Koop BA, Windle JJ, Roodman GD. 2001b. Osteoclasts formed by measles virus infected osteoclast precursors from hCD46 transgenic mice express characteristics of pagetic osteoclasts. Endocrinology 142:2898–2905.

Roodman GD, Kurihara N, Ohsaki Y, Kukita A, Hosking D, Demulder A, Smith JF, Singer FR. 1992. Interleukin-6: A potential autocrine/paracrine factor in Paget's disease of bone. J Clin Invest 89:46–52.

Singer FR. 1999. Update on viral etiology of Paget's disease. J Bone Miner Res 14:29–33.

Siris ES. 1999. Paget's disease of bone. In: Favus MJ, editor. Primer on the metabolic bone diseases and disorders of mineral metabolism. Fourth edition. Philadelphia, PA: Lippincott Williams & Wilkins Press. pp 415–425.

Sparks AB, Peterson SN, Bell C, Loftus BJ, Hocking L, Cahill DP, Frassica FJ, Streeten EA, Levine MA, Fraser CM, Adams MD, Broder S, Venter JC, Kinzler KW, Vogelstein B, Ralston SH. 2001. Mutation screening of the TNFRSF11A gene encoding receptor activator of NF kappa B (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. Calcif Tissue Int 68: 151–155.

Takeshita S, Namba N, Zhao JJ, Jiang Y, Genant HK, Silva MJ, Brodt MD, Helgason CD, Kalesnikoff J, Rauh MJ, Humphries RK, Krystal G, Teitelbaum SL, Ross FP. 2002. SHIP-deficient mice are severely osteoporotic due to increased numbers of hyper resorptive osteoclasts. Nat Med 8:943–949.

Tarquini R, Perfetto F, Tarquini B. 1998. Endothelin-1 and Paget's bone disease: Is there a link? Calcif Tissue Int 63:118–120.

Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, Mumm S. 2002. Osteoprotegerin deficiency and juvenile Paget's disease. New Eng J Med 347:175–184.